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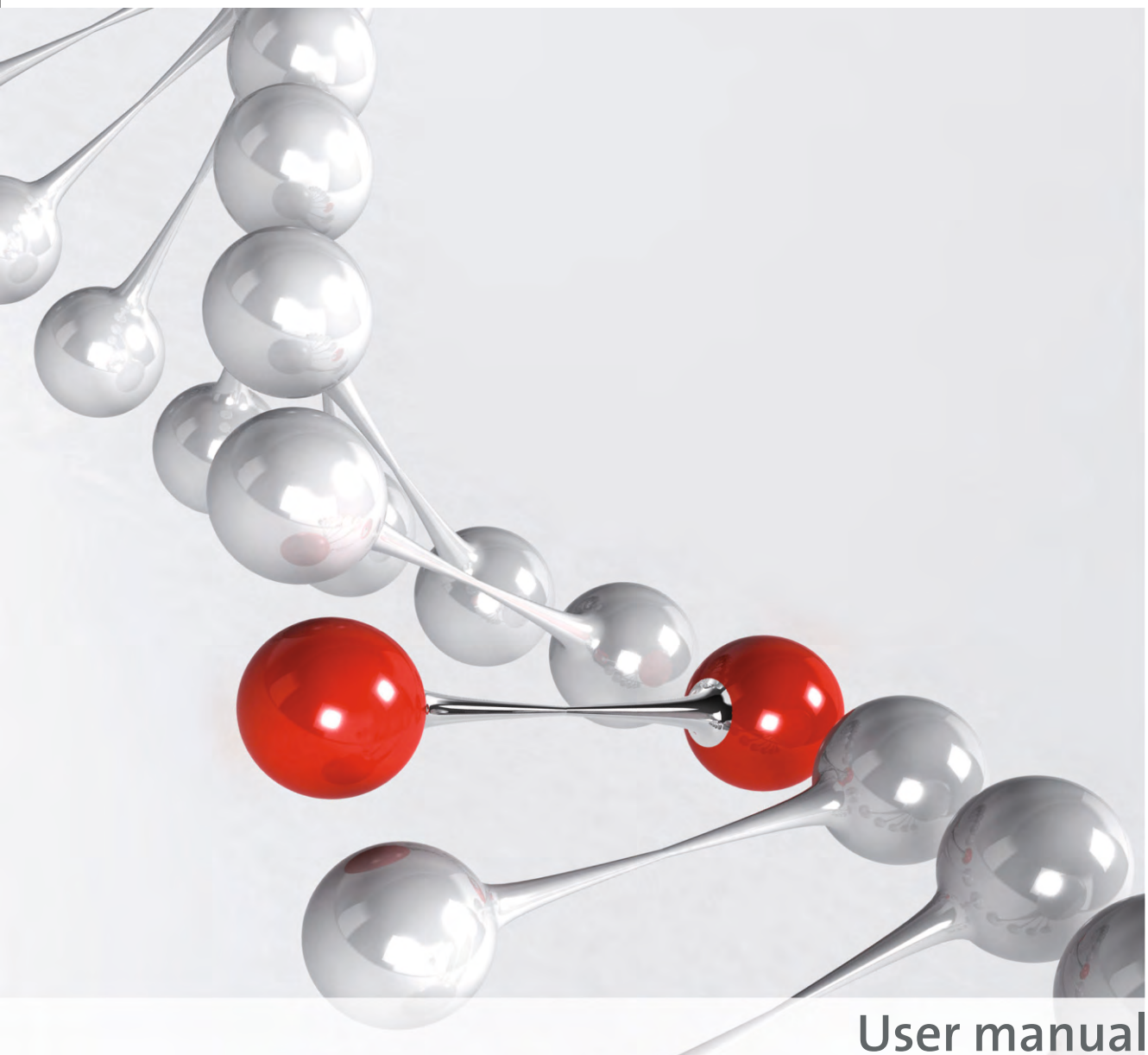

User manual
InviMag® Bacteria DNA Mini Kit/ KFmL
for use on KingFisher® mL, Thermo Fisher Scientific

for automated purification of bacterial DNA from different types of specimen: swabs, tissue, food, paraffin embedded tissue, urine, blood or water samples with magnetic beads

REF 2433150x00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction InviMag® Bacteria DNA Mini Kit/ KFmL

The **InviMag® Bacteria DNA Mini Kit/ KFmL** allows for rapid and economic isolation of high chromosomal bacterial DNA from cell-free body fluids (cerebrospinal fluid, liquor, urine), paper points, bacterial pellets, tissue samples, paraffin embedded tissue, blood, urine, swabs and water using the **InviMag® technology**.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from dried blood stains nor from stool sample, fungi, parasites, or the purification of RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.

Trademarks: InviMag®, RTP®, Thermo, Eppendorf®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

InviMag® and RTP® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Bacteria DNA Mini Kit/ KFmL

Store the **MAP Solution A** at 2-8°C
Store diluted **Proteinase K** at -20°C
Store all other kit components at room temperature

	15 extractions	75 extractions	300 extractions
Catalogue No.	2433150100	2433150200	2433150400
Extraction Tube	15	75	6 x 50
Resuspension Buffer R	15 ml	60 ml	150 ml
MAP Solution A	0.5 ml	2 x 1 ml	7 ml
Binding Buffer B6	3 ml final volume 10 ml	2 x 9 ml final volume 2 x 30 ml	2 x 24 ml final volume 2 x 80 ml
Elution Buffer	2 ml	15 ml	60 ml
Wash Buffer I	7.5 ml final volume 15 ml	2 x 30 ml final volume 2 x 60 ml	2 x 80 ml final volume 2 x 160 ml
Wash Buffer II	18 ml final volume 60 ml	45 ml final volume 150 ml	3 x 60 ml final volume 3 x 200 ml
1.5 ml Receiver Tubes	15	5 x 15	6 x 50
KingFisher ml Tip Combs	3	15	60
KingFisher ml Tube Strips	15	5 x 15	300
Manual	1	1	1
Initial steps	Add 7 ml 99.7% Isopropanol to the **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!	Add 21 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer I**. Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!	Add 56 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!

Kit contents of InviMag® Bacteria DNA Mini Kit/ KFmL w/o plastic

Store the **MAP Solution A** at 2-8°C
Store diluted **Proteinase K** at -20°C
Store all other kit components at room temperature

	15 extractions	75 extractions	300 extractions
Catalogue No.	2433150150	2433150250	2433150450
Extraction Tube	15	75	6 x 50
Resuspension Buffer R	15 ml	60 ml	150 ml
MAP Solution A	0.5 ml	2 x 1 ml	7 ml
Binding Buffer B6	3 ml final volume 10 ml	2 x 9 ml final volume 2 x 30 ml	2 x 24 ml final volume 2 x 80 ml
Elution Buffer	2 ml	15 ml	60 ml
Wash Buffer I	7.5 ml final volume 15 ml	2 x 30 ml final volume 2 x 60 ml	2 x 80 ml final volume 2 x 160 ml
Wash Buffer II	18 ml final volume 60 ml	45 ml final volume 150 ml	3 x 60 ml final volume 3 x 200 ml
1.5 ml Receiver Tubes	15	5 x 15	6 x 50
Manual	1	1	1
Initial steps	Add 7 ml 99.7% Isopropanol to the **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!	Add 21 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer I**. Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!	Add 56 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!
Plastic to be supplied by user (see order information)			
KingFisher ml Tip Combs	3	15	60
KingFisher ml Tube Strips	15	5 x 15	300

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Bacteria DNA Mini Kit/ KFmL,** except **MAP Solution A,** are stable for at least 12 months. **MAP Solution A** is stable for at least 6 months.

All buffers and kit contents of **InviMag® Bacteria DNA Mini Kit/ KFmL**, except **dissolved Proteinase K** and **MAP Solution A**, should be stored at room temperature.

Proteinase K: Dissolved Proteinase K must be stored at -20°C. Dividing the Proteinase K into aliquots and storage at -20°C is recommended.

MAP Solution A: should be stored at 2-8°C.

Wash Buffer: charged with ethanol should be stored at room temperature and should be appropriate sealed. If there are any precipitates within the provided solutions solve these precipitates by careful warming up to room temperature (up to 30°C).

Room temperature (RT) is defined as range from 15 - 30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Bacteria DNA Mini Kit/ KFmL** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Bacteria DNA Mini Kit/ KFmL** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviMag® Bacteria DNA Mini Kit/ KFmL** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany **+49-(0)30-9489-2901/ 2910**
from abroad **+49-(0)30-9489-2907**
or contact your local distributor .

Intended use

The **InviMag® Bacteria DNA Mini Kit/ KFmL** allows for rapid and economic isolation of high quality bacterial DNA from cell free body fluids, paper points, bacterial species, tissue and food samples, paraffin embedded tissue, blood, urine, swabs and water using the **InviMag® technology**.

This kit technology yields genomic DNA from bacteria from different human sources that is free of proteins, nucleases and other impurities and is ready-to-use for different downstream applications like PCR, quantitative PCR, real-time PCR or other routine methods.

For reproducible and high yields appropriate sample storage is essential. The purified DNA is of high quality.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from dried blood stains nor from stool samples, fungi, parasites, or the purification of RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals are only useable once.
Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Bacteria DNA Mini Kit/ KFmL** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Bacteria DNA Mini Kit/ KFmL** to which they apply are listed below as follows:

Wash Buffer I contains a chaotropic salt which is an irritant.

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

Proteinase K (included in the **Extraction Tube**)

danger
H315-319-334-335 P280-305-351-338-310-405

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
in USA : **1 – 800 – 535 – 5053**

Product characteristic of the InviMag® Bacteria DNA Mini Kit/ KFmL

Starting material	Yield	Time	Ratio
max. 10^9 bacteria 1-10 mg tissue, biopsy material paraffin embedded tissue 1-100 µl whole blood 200 µl cell-free body fluid 15-50 ml urine paper points swabs up to 1 l water	depends on the starting material	approx. 20-25 min after lysis	A_{260} : A_{280} 1,7 – 2,0

The **InviMag® Bacteria DNA Mini Kit/ KFmL** allows for rapid and economic isolation of high quality genomic DNA from a broad range of bacteria species (up to 10^9 bacteria cells) and different sources, using the **RTP® technology** and a KingFisher ml workstation.

For the wide range of different samples the **InviMag® Bacteria DNA Mini Kit/ KFmL** includes protocols adapted to the need of every kind of starting material. All types of samples are transferred into the **Extraction Tube** together with a specially designed **Resuspension Buffer R.** The prefilled buffer and enzymes lyse the samples, stabilize nucleic acid and enhance the selective DNA adsorption to the beads. Mostly the lysis is carried out at two different incubation temperatures in the **Extraction Tube** to increase the sensitivity. In addition to the rigorous lysis procedure, simple pretreating steps have been introduced, ideally for purification of genomic DNA, especially from gram positive bacteria of different sources followed by binding of the bacterial nucleic acids to the InviMag® Beads, washing steps and final elution.

High extraction efficiency and detection sensitivities are realized.

The “hands-on time“ necessary for the whole procedure is reduced to a minimum.
The kit technology yields bacterial DNA from different human samples that is free of proteins, nucleases, and other impurities and ready to use for downstream application like PCR*, quantitative PCR, real-time PCR or other routine laboratory methods.

The procedures require minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are optimized to avoid sample-to-sample cross-contamination.

No toxic or hazardous chemicals like phenol/chloroform or ß-Mercaptoethanol are used.
Traditional time-killing procedures can be replaced using the **InviMag® Bacteria DNA Mini Kit/ KFmL.**

Due to the high purity, the isolated total DNA is ready-to-use for a broad panel of downstream applications (see below) or can be stored at -20°C for subsequent use.

- PCR*
- Real-time PCR (quantitative PCR, like TaqMan und LightCycler technology)
- Mircoarray application
- RFLP-Analysis

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **InviMag® Bacteria DNA Mini Kit/ KFmL** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principle and procedure

The **InviMag® Bacteria DNA Mini Kit/ KFmL** procedure comprises following steps:

- lysis of sample material
- binding the genomic DNA to the magnetic beads
- washing the beads and elimination of waste and ethanol
- elution of total DNA

This manual contains 9 protocols.

The samples are lysed in an optimized buffer and enzyme mixture. The lysates are transferred to the subsequent automatic purification procedure based on magnetic beads. The DNA binds to magnetic particles, followed by washing steps and the final elution. The purified high quality DNA is ready-to-use for subsequent downstream applications like PCR amplification, quantitative PCR, real-time PCR or other routine laboratory methods.

Sampling and storage of starting material

Pathogens (*Lysteria* ssp.) in food material

For the detection of bacteria (*Lysteria*) in foods an enriched and cultivation following the EU regulations and § 35 of the food law ios required. An aliquot of the culture is used and the bacteria will be centrifuged after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA.

Bacterial cultures

Bacterial cultures grow in the presence of a selective agent such as an antibiotic. The yield and quality of the DNA depends on factors such host strain, inoculation, antibiotic, and type of culture medium. The bacteria will be centrifuged after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA.

Paper points

Paper points can be stored at room temperature for up to 6 hours. For long-term storage, we recommend dry storage at 2-8°C.

Blood

Blood samples can be stored at room temperature for up to 6 hours or at 2-8°C for up to 24 hours. For long term storage, we recommend freezing samples at –20°C or –80°C. Multiple thawing and freezing cyckes before isolating the DNA should be avoided. If cryoprecipitates (formed during thawing of frozen samples) are visible, avoid aspirating them. The amount of purified DNA from max. 100 µl whole blood depends on the white blood cell content of each blood sample. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples.

Tissue/ biopsy material

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA. Use of poor quality starting material also leads to reduced length and yield of purified DNA. The thawing process should be performed in the Extraction Tube. The amount of purified DNA from max. 10 mg tissue sample depends on the nature of starting material.

Urine
The bacteria must be centrifuged and the supernatant completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh pellets or frozen pellets. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA. The amount of purified DNA from max. 15 - 50 ml urine depends on the bacterial titer.

Swabs, saliva
The protocol works with fresh saliva, prepared swabs as well as with dried swabs. Please note, that DNA from stored and dried swabs are often characterized by isolation of apoptotic DNA (visible on agarose gel as typical apoptotic DNA banding pattern).The protocol has not been validated for isolation of DNA from swabs which are stored in special storage buffers from other providers.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Sample preparation

Bacteria must be cultivated at special conditions. An aliquot of the bacterial suspension is used to get a bacterial pellet by centrifugation at high speed for 5 min. The supernatant is dicarded.

Lysis
Samples are lysed at non-chaotropic conditions with different elevated temperatures while continuously shaking. Lysis is performed in the presence of Lysozyme to break the cell wall of the bacteria, **Lysis Buffer** to lyse the cells and **Proteinase K** to digest the proteins. Every component is provided prefilled in the **Extraction Tube.** Unlysed sample parts should be removed before the binding step.

Binding genomic DNA
By adding **Binding Buffer B6** and the **MAP Solution A** to the lysate, optimal binding conditions are adjusted. The DNA is adsorbed to the InviMag® beads as the beads are mixed carefully by the magnetic rods.

Removing residual contaminants
Contaminants are efficiently removed using two different **Wash Buffers** while the bacterial, genomic DNA remains bound to the beads.

Elution of pure genomic DNA
Genomic DNA is eluted from the beads using 100 µl **Elution Buffer**. The eluted DNA is ready-to-use in different downstream applications.

Equipment and reagents to be supplied by user

- Eppendorf Thermomixer
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipet with tips
- 96 – 100 % ethanol
- ddH_2O
- optional octane
- Isopropanol*

*The **InviMag® Bacteria DNA Mini Kit/ KFmL** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important notes

Important points before starting a protocol

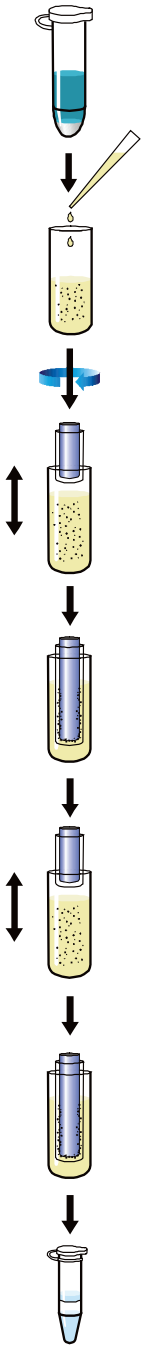
Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps should be carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

15 extractions:
Add 7 ml 99.7% Isopropanol to the **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Mix shortly and keep the bottle always firmly closed. Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II**. Mix shortly and keep the bottle always firmly closed.
75 extractions:
Add 21 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer I**. Mix shortly and keep the bottle always firmly closed. Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**. Mix shortly and keep the bottle always firmly closed.
300 extractions:
Add 56 ml 99.7% Isopropanol to each **Binding Buffer B6.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol each the bottle **Wash Buffer I**. Mix shortly and keep the bottle always firmly closed. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**. Mix shortly and keep the bottle always firmly closed.

Scheme of the InviMag® Bacteria DNA Mini Kit/ KFmL



Please read protocols prior the start of the preparation carefully

Please prepare the sample preparation outside the KingFisher mL platform following the instructions:
During lysis, prefill the KingFisher Tube strips with required buffers and appropriate volumes

Tube A: 400 µl **Binding Buffer B6** and 20 µl **MAP Solution A**
Tube B: 800 µl **Wash Buffer I**
Tube C: 800 µl **Wash Buffer II**
Tube D: 800 µl **Wash Buffer II**
Tube E: 200 µl **Elution Buffer**

Lysis of starting material in the Extraction Tube
Add required amount of **Resuspension Buffer R** to the sample and transfer the sample to the Extraction Tube
Vortex for 5 s.
Incubation in a Thermomixer at 65°C for 10 min (continuously shaking increases the lysis efficiency).

Transfer of lysate to InviMag® beads and **Binding Buffer B6** *(follow preparing instructions)*in the 5-tube strip format

DNA binding to the magnetic beads

Magnetic separation

Washing

Magnetic separation

Elution and magnetic separation
Pure DNA

Protocol 1: Isolation of bacteria from cell free body fluids (serum, plasma, cerebrospinal fluid, liquor)

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 10 min in a thermomixer
Mix 200 µl Resuspension Buffer R with 200 µl of liquid sample, transfer the sample to the Extraction Tube and vortex for 5 s.

Incubate the sample in a thermomixer at 65°C for 10 min (continuous shaking increases the lysis procedure).

During lysis, see under "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube with the lysis mixture into a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer approximately 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17

Protocol 2: Isolation of DNA from periodonthopathogenic bacteria from paper points

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 10 min in a thermomixer
Transfer the paper point into the Extraction Tube and add 400 µl Resuspension Buffer R to the Extraction Tube. Vortex for 5 s.

Incubate the sample in a thermomixer at 65°C for 10 min (continuously shaking increases the lysis procedure).

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube with the lysis mixture into a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app. 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program "InviMag Bacteria KFmL", see instructions on page 17

Protocol 3: Isolation of DNA from bacteria pellets (1 x 10^9 bacteria cells)

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 10 min in a thermomixer
Pellet the bacteria by centrifugation. Resuspend the bacterial pelltet in 400 µl Resuspension Buffer R. Transfer the resuspended sample into the Extraction Tube and vortex for 5 s.

Incubate the sample in a thermomixer at 65°C for 10 min (continuously shaking increases the lysis efficiency).

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 10) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube with the lysis mixture into a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app. 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17

Protocol 4: Isolation of DNA from swabs or 200 µl rinsed liquid from swabs

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 10 min in a thermomixer
Place the swab into the Extraction Tube and add 400 µl of Resuspension Buffer R. Vortex for 5 s.

Place the Extraction Tube tube in a thermomixer and incubate while continuously shaking for 15 min at 65°C.

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube with the lysis mixture in a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app. 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17.

Important Note: *To get the maximum yield of bacterial DNA it is essential to keep the swab inside the reaction tube during lysis. It is possible to cut the shaft of the swab, so that you the cap of the Extraction Tube can be closed. It is also possible to perform the lysis step with an opened cap. The removing of the swab from the Extraction Tube ahead of time will be lead to a dramatically reduced final yield! After lysis, carefully squeeze out the swab inside the tube wall and discard the swab.*

Protocol 5: Isolation of bacterial DNA from tissue biopsies

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 30–60 min in a thermomixer
Transfer 1-10 mg of the biopsy material into the Extraction Tube and add 400 µl Resuspension Buffer R. Close the cap and vortex for 5 s.

Place the Extraction Tube in a thermomixer and incubate for 30–60 minutes at 65°C while continuously shaking. Lysis time can be increased if the lysis step is not complete.

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a Thermomixer
Place the Extraction Tube into a Thermomixer and incubate at 95°C for 10 min (continuous shaking increases the lysis efficiency). After lysis centrifuge the sample at max. speed for 1 min to spin down unlysed material.

DNA Binding
After centrifugation transfer app. 450 µl of the supernatant into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17.

Protocol 6: Isolation of bacterial DNA from paraffin embedded tissue

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Deparaffination
Transfer the sample into a 1.5 ml reaction tube. Add 1 ml octane and vortex carefully to dissolve the paraffin. Follow the dissolution until the tissue sample looks transparent (while paraffin is still white). Centrifuge for 2 min at maximum speed to collect the tissue sample. Discard the supernatant very careful *(This step should be repeated if paraffin remains are still present in the sample).* Add 0.5 ml 96% ethanol to the tissue sample and mix the tube thoroughly. Centrifuge shortly and remove the ethanol by aspiration with a pipette. Then incubate the open tube at 56°C to evaporate residual ethanol.

Lysis at 65°C for 30–60 min in a thermomixer
Transfer the deparaffinized tissue sample into the Extraction Tube and add 400 µl Resuspension Buffer R. Close the cap and vortex for 5 s.
Place the Extraction Tube in a thermomixer and incubate for 30–60 min at 65°C while continuously shaking. Lysis time can be increased if the lysis is incomplete.
During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube in a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency). After lysis centrifuge the sample at max. speed for 1 min to spin down unlysed material.

DNA Binding
After centrifugation, transfer app. 450 µl of the supernatant into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17.

Protocol 7: Isolation of bacterial DNA from urine samples

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Sample Collection
Centrifugation of the collected urine sample (15–50 ml) for 15 min at 1.300 x *g* (3500 rpm). Decant the supernatant carefully and resuspend the pellet with 3 ml of 1x PBS. Centrifuge for 5 min at 1.300 x *g* (3500 rpm). Decant the supernatant carefully.

It is important to remove the supernatant completely! Residual amounts of liquid will have a negative influence on the further extraction procedure

Lysis at 65°C for 10 min in a thermomixer
Resuspend the pellet in 400 µl Resuspension Buffer R. Transfer the resuspended cells into the Extraction Tube and vortex for 5 s.

Incubate the sample in a thermomixer at 65°C for 10 min (continuously shaking increases the lysis efficiency).

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube with the lysis mixture in a thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app. 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A.

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5.) Start the program, see instructions on page 17.

Protocol 8: Isolation of bacterial DNA from blood samples

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Lysis at 65°C for 20 min in a thermomixer
Mix up to 100 µl of whole blood with 300 µl Resuspension Buffer R
(If the starting volume of whole blood is lower than 100 µl add more Resuspension Buffer R. The final volume schould be 400 µl). Transfer the sample completely into the Extraction Tube and close the cap. Vortex for 5 s.

Place the Extraction Tube in a thermomixer and incubate for 15 min at 65°C while continuously shaking.

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a Thermomixer
Place the Extraction Tube into a Thermomixer and incubate at 95°C for 10 min (continuously shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl of Binding Buffer B6 and 20 µl MAP Solution A (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

Start the program, see instructions on page 17.

Protocol 9: Isolation of bacterial DNA from water samples (no waste water)

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer B6*** *– see instruction page: 11*

Attention Please*:* *STRATEC Molecular offers on request also a very special protocol for isolation of DNA from Legionella species from 1 L water samples*

Sample Collection
Concentrate the starting sample (e.g. 1 L) by standard procedures like centrifugation or filtration. Finally spin down the bacteria by centrifugation. Decant the supernatant carefully and resuspend the sediment in 10 ml of 1x PBS. Centrifugation for 5 min at 3500 rpm. Decant the supernatant carefully.

Lysis at 65°C for 10 min in a thermomixer
Add 400 µl Resuspension Buffer R to the sediment and resuspend the pellet by pipetting up and down. Transfer the resuspended sample into the Extraction Tube, close the cap and vortex for 5 s.

Incubate the sample in a thermomixer at 65°C for 10 min (continuously shaking increases the lysis efficiency).

During lysis, see "Preliminary Steps to process the sample onto the KingFisher System" (page 17) and follow the instructions.

Lysis at 95°C for 10 min in a thermomixer
Place the Extraction Tube in a thermomixer and incubate at 95°C for 10 min (continuous shaking increases the lysis efficiency).

DNA Binding
After lysis, transfer app. 450 µl of the lysed sample into the Tube A of the KingFisher tube and add 400 µl Binding Buffer B6 and 20 µl MAP Solution A.

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5.) Start the program, see instructions on page 17.

Preliminary steps to process the sample onto the KingFisher system

1. During sample lysis, prefill the tubes of the KingFisher tube strips with the required buffers and appropriate volumes.

KingFisher ml Tube Strip Setup:

Tube A: 400 µl **Binding Buffer B6** and 20 µl **MAP Solution A**

Note: *It is important to mix the bottle with MAP Solution A carefully by vigorously shaking or vortexing!*

Tube B: 800 µl Wash Buffer I

Tube C: 800 µl Wash Buffer II

Tube D: 800 µl Wash Buffer II

Tube E: 200 µl Elution Buffer

2. Insert the filled KingFisher tube strips into the KingFisher System on the right position !

3. Insert the KingFisher tip comb into the instrument!

The following extraction steps run automatically on the KingFisher system!

Start the program “InviMag Bacteria KFmL”

Important Notes :
1. After finishing the extraction protocol, the Tube E contains the extracted DNA.
Store the DNA under adequate conditions.
We recommend transferring the extracted DNA into 1.5 ml receiver tubes for further storage and the freeze the DNA at -20°C.

2. If the extracted DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube and centrifuge at maximum speed for 1 min and transfer the DNA containing supernatant into a new tube.

Automatic extraction steps running on the KingFisher system

1. Binding of the DNA
Automatically sample mixing for 5 min. MAP separation.
Moving of the MAP into the Tube B.

2. First Washing
Automatically sample mixing for 2 min. MAP separation. Moving of the MAP into the Tube C.

3. Second Washing
Automatically sample mixing for 1 min. MAP separation. Moving of the MAP into the Tube D.

4. Third Washing and Drying
Automatically sample mixing for 1 min. MAP separation. Drying the MAP outsight the Tube for 5 min. Moving of the MAP into the tube E.

5. Elution of the DNA
Incubation of the MAP into the Tube E for 5 minutes by mixing. MAP separation.
The MAP will than automatically removed into the well D (disposal).

The extracted DNA can now be transferred into 1.5 ml receiver tubes.
Optional: Carryover of magnetic particles should be removed by centrifugation at max. speed for 1 min. Transfer the cleared eluate into a new tube.

For self programming of the KingFisher ml system (program “InviMag Bacteria KFmL”)

[Protocol Properties]

Name = InviMag Universal Bacteria Kit/ KFmL
Protocol template version = 3.1
Instrument type = KFmL
Description = KFmL protocol for isolation of bacterial DNA from liquid samples with the InviMag Bacteria DNA Kit/ KFmL.

[Layout Data]

A (Binding):
Plate type: KingFisher tubestrip 1000 ul
Reagents:
Name: Lysed sample
Volume [µl]: 450
Type: Sample

Name: Binding Buffer B6
Volume [µl]: 200
Type: Reagent

Name: MAP Solution A
Volume [µl]: 20
Type: Reagent

B (Washing 1):
Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer I
Volume [µl]: 600
Type: Reagent

C (Washing 2)
Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer II
Volume [µl] :800
Type: Reagent

D (Washing 3)
Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer II
Volume [µl]: 800
Type: Reagent

E (Elution)
Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Elution Buffer
Volume [µl]: 200
Type: Reagent

[Steps Data]

Tip1: (Tip KingFisher ml tip comb)

Binding: (Plate Bacteria (A) – Binding(
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Wash1: (Plate Bacteria (B) - Washing 1)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:02:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash2: (Plate Bacteria (C) - Washing 2)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash3: (Plate Bacteria (D) - Washing 3)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Drying: (Plate Bacteria (D) - Washing 3)

Dry time [hh:mm:ss]: 00:05:00
Tip position: Outside well / tube

Elution (Plate: Bacteria (E) – Elution)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Bead Removal: (Plate Bacteria (D) - Washing 3)
Release time [hh:mm:ss]: 00:00:30
Release speed:Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA	insufficient lysis	increase lyses time, but prevent too long lyses tome because this also decrease yield reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer**, be sure you pipet the **Elution Buffer** with the right amount to the right position
	low amount of **MAP Solution A**	mix **MAP Solution A** thoroughly before pipetting to the KingFisher tube
low concentration of extracted DNA	too much **Elution Buffer**	elute the DNA with lower volume of **Elution Buffer**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
degraded or sheared DNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20°C)! avoid thawing and freezing of the material old material often contains degraded DNA
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher Software 3.1
The KingFisher Software 3.1 was used to create assay files for the KFmL, KF96 and KFflex96 instruments. The respective assay file can either be transferred onto the KingFisher workstation or be started directly from within the BindIt software. Keep in mind that directly run assay files are not stored in the workstation memory!

Note: When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.1 for assay development as this software version includes the correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 workstations to ensure the best purification result.

PC requirements for KingFisher Software 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 MHz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA require careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure it will function well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store genomic DNA at 2-8°C. Storing genomic DNA at -20°C may cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNA can be stored at 2-8°C or at -20°C.

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at 2-8°C. Minimize vortexing of genomic DNA because this may cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid DNA and other small.

Ordering information

Product	Package size	Catalogue No.
InviMag® Bacteria DNA Mini Kit/ KFmL	15 extractions	2433150100
InviMag® Bacteria DNA Mini Kit/ KFmL	75 extractions	2433150200
InviMag® Bacteria DNA Mini Kit/ KFmL	300 extractions	2433150400
InviMag® Bacteria DNA Mini Kit/ KFmL (wp)	15 extractions	2433150150
InviMag® Bacteria DNA Mini Kit/ KFmL (wp)	75 extractions	2433150250
InviMag® Bacteria DNA Mini Kit/ KFmL (wp)	300 extractions	2433150450

Single components

Extraction Tube	10 tubes	7433301500
MAP Solution A	1 ml	7433305100
Resuspension Buffer R	30 ml	7433302200
Binding Buffer B6	30 ml	7433302100
Wash Buffer I	30ml	7433303500
Wash Buffer II	18 ml	7433303600
Elution Buffer	15 ml	7433304000
KingFisher ml Tip Comb	1 piece	0030014400
KingFisher ml Tube	1 piece	0030014500

Ordering information (KingFisher mL and consumables from Thermo Scientific)

Cat.no	Description
5400050	KingFisher mL, Magnetic Particle Processor, 100-240 V, 50/60 Hz
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20x45 pcs)
97002131	KingFisher mL Combi 60 (tubes and tip combs for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tip combs for 240 samples)

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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